microscope based single-molecule force spectroscopy (SMFS) on structurally homologous proteins - ubiquitin, SUMO1, and SUMO2. SUMOs are found to be mechanically weaker than ubiquitin, in spite of their high structural homology, when pulled along N-C direction [1]. The unfolding forces of these proteins follow the same trend as the number of sidechain contacts within them. The energy landscape parameters reveal that SUMOs are more flexible than ubiquitin. We have performed steered molecular dynamics simulations on these proteins to gain atomistic insights into their unfolding pathways. The hydrogen bonds in the mechanical clamp between the terminal β-strands of these proteins are ruptured at the transtion state and proteins unfolded in an all-or-none fashion. The interactions between the β2 strand and the α-helix also weaken at the transition state. We examine the different types of inter-resiude interactions in these proteins to rationalize the lower mechanical stability of SUMOs relative to ubiquitin. Role of water penetration during mechanical unfolding was also investigated.
We have further studied the effect of ligand binding on the mechanical stability of SUMO proteins using SMFS in presence of short peptides derived from the binding motifs of SUMO targets. The unfolding force of SUMO1 increased from ~130pN to ~170pN upon ligand binding. Dependence of the unfolding force on the pulling speed was higher for SUMO1 bound to the peptides than the unbound protein. The flexibility of SUMOs decreased upon ligand binding suggesting a possible role of SUMO flexibility in SUMOylation.
[1] Kotamarthi HC, Sharma R, Ainavarapu SRK, Biophys. J (2013), *104,* p2273.

1973-Pos Board B703
Direct Observation of the Time Evolution of a Biomolecular Transition
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Fundamental to our current understanding of biomolecular transitions is the notion of diffusion on a free energy landscape, which serves as a model for processes ranging from protein and RNA folding to the stepping of molecular motors. Distilling the complex interactions of biomolecules into a single transition coordinate across a free energy landscape has led to many accurate predictions. However, such motions have never been observed directly until now. Here, we present an analysis of thousands of nucleosome state transitions, observed at different forces and ionic concentrations, using an optical trap and associated data acquisition with microsecond time resolution. These measurements permit us to directly observe the time evolution across the transition path and the distribution of transition path times. We furthermore show how these properties are affected by force and ionic concentration.

1974-Pos Board B704
Mechanically Tightening a Protein Slipknot into a Trefoil Knot
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Knotted polypeptide chain are found to be one of the most surprising topological features in some proteins. The topological difficulty of the folding of knotted proteins has become a challenging problem. It was reported that a structure of slipknot could serve as an important intermediate state during the folding of knotted proteins. Here we use single molecule force spectroscopy (SMFS) as well as steered molecular dynamics (SMD) simulations to transform a slipknot protein AFV3-109 into a tightened trefoil knot. Our results show that by pulling on the N-terminus and the threaded loop of AFV3-109, the protein can be unfolded via multiple pathways and the slipknot would be transformed into a tightened trefoil knot with ~13 amino acid residues involved because the polypeptide chain is apparently shortened by ~4.7 nm. The SMD simulations agree with our experiments and provide detailed molecular mechanism of mechanical unfolding and knot tightening of AFV3-109. Interactions between shearing β-strands on threaded loop and knotting loop provide high mechanical resistance in the process of forming the trefoil knot, i.e., pulling threaded loop through knotting loop.

1975-Pos Board B705
Large-Scale Molten-Globule Dynamics Contribute to Titin Contractility
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When exposed to high forces, titin unfolds in discrete steps via unfolding of its globular domains. By contrast, refolding is strongly inhibited by mechanical force and chain extension, which results in a large force hysteresis in stretch-relaxation cycles. Whereas the stretch force curve of titin is populated by sawtooth-shaped transitions, the relaxation force curve is devoid of significant transitions, making it difficult to capture the refolding event. Although titin refolds if incubated in the contracted state, the exact trajectory of the folding process is unclear. To explore the detail of titin's mechanically-driven folding and unfolding, we manipulated single molecules with high-resolution optical tweezers. Whereas titin extended in discrete steps at high constant forces, after quenching the force to low levels the extension fluctuated without resolvable discrete events but with low frequency (second timescale) and high (several 100 nm) peak-to-peak amplitude. In constant-trap-position experiments at very low (<1 pN) average forces fluctuations were observed, suggesting that the domains hop between an extensible unfolded and a compact molten-globule state. Monte-Carlo simulations based on a compact molten-globule intermediate recovered all features of the force-clamp results. Under mild denaturing conditions (0.5 M urea) that favor the molten-globule state, the length and force fluctuations appeared even in constant-velocity experiments, indicating that this intermediate is part of the folding trajectory. Because the transition from the unfolded to the molten-globule state shortens the chain faster than a purely entropic collapse, an additional sarcomeric contractility may arise under stressed conditions when titin's domains become unfolded.

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Halotag Tethers to Study Titin Folding at the Single Molecule Level
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Single molecule force spectroscopy techniques have become important tools to study properties of proteins that operate under force; a common occurrence in Biology. Lack of specific attachment of single molecules to the force probe limits the measuring time and often leads to tethering at random places along a protein substrate. Here we present a technique based on polyprotein engineering and HaloTag attachment that is capable of avoiding these limitations. This method shows full-length polyprotein unfolding, high pick-up yield and detachment forces of ~2 nN. Compared to other covalent attachments, this method shows a specific signature, given by the partial unfolding of HaloTag. We find that placing the HaloTag at the N-end of the construct shows an unfolding contour length of 66 nm and a mechanical strength of ~131 pN. Placing the HaloTag at the C-end of the construct exposes to force a more stable part of the protein, which shows an increased mechanical strength of ~491 pN and a contour length increment of 27 nm. We use HaloTag covalent attachment to study the folding of I27, a model protein from human titin. We expose I27 polyprotein constructs to successive cycles of high and low force, which unfolds and refolds the component protein domains. Covalent attachment greatly expands the tethering time of a polyprotein construct and allows for the measuring of the unfolding and folding rates from a single trace. Furthermore, repeated unfolding and refolding of the same polyprotein reveals subtle effects such as folding intermediates and slow oxidation of cysteines, which affects the mechanical stability of titin. This method opens a new approach to study protein folding at the human timescale, where proteins such as titin are slowly turned over after several days and to determine their energy landscape.

Advances in Single-Molecule Spectroscopy II

1977-Pos Board B707
Computational Analysis of the Single Molecule AFM Force Spectroscopy Data
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AFM force spectroscopy is a widely applied to various molecular systems providing an important information on the strength of intramolecular and intermolecular interactions. However, a structural characterization of the systems is limited by the lack of theoretical approaches capable of direct comparison with experiment. Steered Molecular Dynamic (SMD) simulation is widely used for modeling of the AFM pulling experiments, but it is typically performed at pulling rates 5 nm/ns which are 10^7 times higher than those for typical experimental conditions. Here we describe the computational approach utilizing Monte Carlo Pulling (MPC) simulation enabling us to model AFM pulling experiments at conditions corresponding to experimental ones. Tested by the comparison with experimental data for titin I-band(PDB ID: 1TIT), the approach was applied to modeling of the AFM force spectroscopy experiments on probing of interaction of misfolded peptides CGNNQQNY and Aβ14-23 within dimers. The structures of the CGNNQQNY dimers were obtained from the Replica Exchanged MD simulations. The results for antiparallel out-of-register β sheet of CGNNQQNY produce the force ~100 pN which is in line with the